

# DWMax experimental data

## History for shaking culture with Deep well plate

Deep well plate (hereinafter, DWP) is a container originally used to store samples. However, when the method of High-throughput screening (HTS) was developed around the year 2000, it started being used not only for Storage but also for other applications such as Cultivation and Reaction. At that time, we received a customer's inquiry "Do you have any idea on how to perform a shaking culture with DWP effectively?" Thereafter, we managed to develop the "Bioshaker"® MBR-024 for DWP," which was the predecessor of the current Bioshaker®.

### USER'S VOICE

I was really amazed that an effective shaking culture of heavy yeast in such a small well of the Deep well plate can be realized!

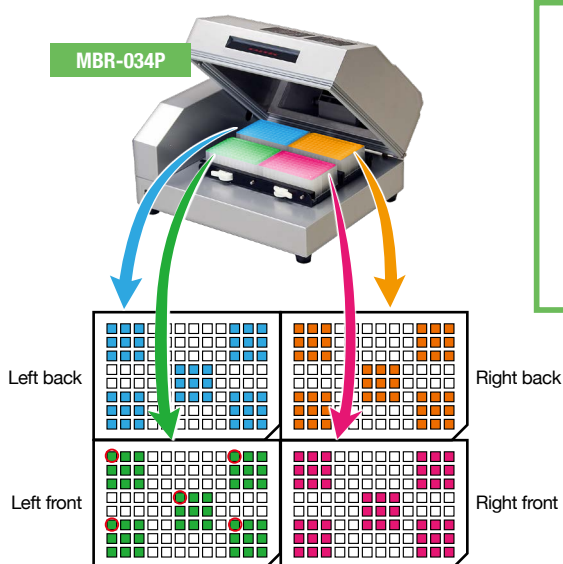


The verification for the mounting position and the variation among the wells and the effectiveness of shaking culture of yeast with 96-DWP.

## Validation for New Max drive. To reduce the variations due to the position of vessels and wells.

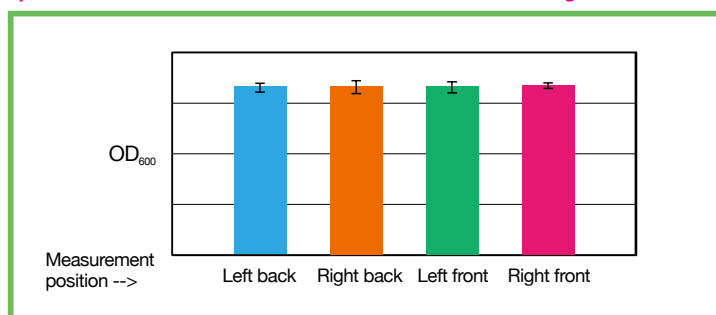
We verified the variation in turbidity ( $OD_{600}$ ) for the yeast in four wells out of 96-deep well plate (squared well). Since yeast has a larger cell than *E. coli*, it is inclined to sink to the bottom of the well, in particular, the shaking culture with DWP if the stirring effectiveness is poor, which causes the variation in turbidity. At the same time, we also compared the popular shaking culture method using an Erlenmeyer flask to 96-deep well plate in turbidity ( $OD_{600}$ ). The variation among the positions of deep wells are indicated in the bar graph. The deviation of each well plate in the same DWP is indicated in error bars in black (Fig.1). As a result, each well plate had no big difference in deviation. We have concluded that the stirring effectiveness at any positions in DWP made it uniform. We then compared it with the Erlenmeyer flask in turbidity, and finally a doubled value ( $OD_{600}$ =above 8) of the upper value in the Erlenmeyer flask was obtained (Figure.2).

### [Measurement position of $OD_{600}$ ]

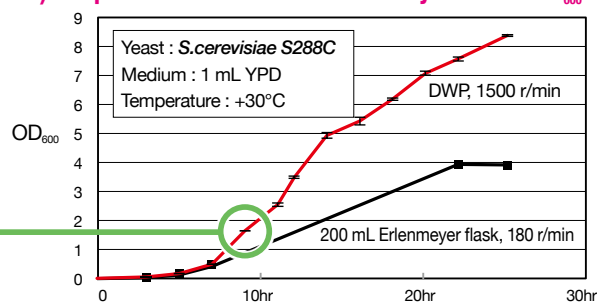


The six wells per group were respectively allocated at the four corners and at the center of DWP. Measured the five deep wells allocated among from respectively five groups at the four corners and at the center of DWP. \*The deep well that is circled in red is measured for variation in the Front Left, in addition to the Left back, Right front, and Right back.

### 1) Variations were measured for 9 hours since the shaking culture started.



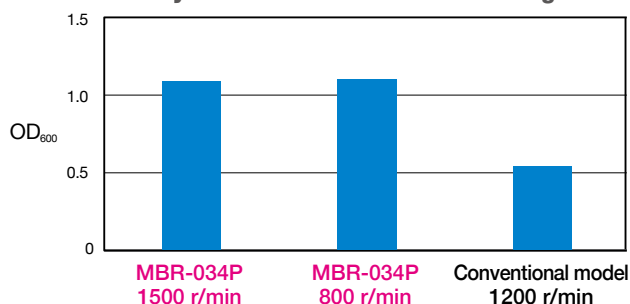
### 2) Comparison between DWP and Erlenmeyer flasks in $OD_{600}$ .



## Comparison with the conventional model: Shaking culture of *E. coli* with 96-well plate

We made shaking culture of *E. coli* using MBR-034P and the conventional model with 96-well plate for comparison. 4 hours after the start of shaking culture, we measured the turbidity. As a result, we discovered that  $OD_{600}$  of MBR-034P reached " $OD_{600}=1.0$ " in half the time earlier than the conventional model, even at a slower shaking speed.

### Turbidity 4 hours after the start of shaking culture



[Conditions] \*All models in common

- 96-deep well plate: Square hole (conical bottom), Capacity approx. 2 mL per well
  - Sealing: Gas Permeable Adhesive Seals
  - Cultivation volume: 1 mL (approx. 50% of the well volume is optimal for aeration).
- > In the case of 1 mL, the solution does not reach the sealing when shaking up to 1500 r/min, but reaches the sealing at 1600 r/min.